HHS Public Access

Author manuscript

J Expo Sci Environ Epidemiol. Author manuscript; available in PMC 2019 May 23.

Published in final edited form as:

J Expo Sci Environ Epidemiol. 2018 March ; 28(2): 193–201. doi:10.1038/jes.2017.10.

In vitro human skin permeation of benzene in gasoline: Effects of concentration, multiple dosing and skin preparation

H. Frederick Frasch and **Ana M. Barbero**

Health Effects Laboratory, National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA.

Abstract

In vitro human skin benzene permeation was measured from gasoline formulations with benzene concentrations ranging from 0.8 to 10 vol% and from neat benzene. Steady-state fluxes (J_{SS}), permeability coefficients (k_p) and lag times (t_{lag}) were calculated from infinite dose exposures. Permeation of benzene from small gasoline doses administered over a two-day period was also studied. The thermodynamic activity of benzene in gasoline at 30°C was determined and the solution is near-ideal over the range from 0.8 to 100 vol%. J_{SS} through human epidermal membranes were linear (R^2 = 0.92) with concentration over the range from 0.8 to 10 vol %. J_{SS} (μg/cm^2/h) from gasoline (0.8 vol% benzene = 6.99 mg/ml) through epidermis and full-thickness skin were 9.37 ± 1.41 and 1.82 ± 0.44, respectively. Neat benzene J_{SS} was 566 ± 138. Less than 0.25% of the total applied benzene mass from finite doses (10 μl/cm^2) of gasoline was detected in receptor cells, and a small reduction of barrier function was observed from six total doses administered over 2 days. Application of these results to dermal exposure assessment examples demonstrates a range of systemic benzene uptakes that can be expected from occupational and consumer dermal exposures to gasoline, depending on the type and extent of exposure

Keywords

activity coefficie; dermal risk assessment; finite dose; fractional absorption; infinite dose; lag time

INTRODUCTION

Benzene exposures have been linked to various well-described acute and chronic health effects and benzene has been recognized as a human carcinogen by various international agencies for over 30 years.[1–3] The US EPA[1] classifies benzene as a “known” human carcinogen (Category A) for all routes of exposure, including the dermal route.

Benzene penetrates the skin following dermal contact. Dermal absorption studies have been reported using neat benzene, aqueous benzene and benzene in various organic mixtures, including gasoline (summarized by Williams et al.[4]). These studies demonstrate the capacity

Correspondence: Dr H. Frederick Frasch, Health Effects Laboratory, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, WV 26505, USA. Tel.: +1 304 285 5755. Fax: +1 304 285 6041. HFrasch@cdc.gov.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

to which benzene penetrates the skin and is systemically absorbed. Reported average human *in vitro* steady-state flux (J_{ss}) measurements of neat benzene range from 99 (Lodén[5]) to 1855 μg/cm^2/h (Blank and McAuliffe[6]). This nearly 20-fold range in measured values points to a need for additional data, and possible methodological issues in both studies can be identified. The study by Lodén[5] used full-thickness skin, which is not recommended for lipophilic compounds,[7] in a flow-through diffusion cell system in which receptor medium is collected by dripping into open vials in a fraction collector. Vapor losses are to be expected in this open system; however, the author took steps to minimize losses and claimed they "did not exceed 5%" but did not provide data, and benzene freely permeates through polyethylene tubing[8] that was used by Lodén. Nevertheless, the data show excellent steady-state linearity up to 16 h of exposure. The large human epidermis measurement reported by Blank and McAuliffe[6] has been called into question[4,9,10] based on perceived inconsistencies in the reported value. Other human flux measurements have been summarized by Williams et al.,[4] who concluded that a range of neat benzene fluxes from 200 to 400 μg/cm^2/h is supported. Additional data would be beneficial to add to the weight-of-evidence on neat benzene dermal uptake rates.

Thus there is ample evidence that benzene permeates skin, even if a definitive penetration rate of neat benzene is not universally agreed upon. There is less research on the permeation of benzene from hydrocarbon mixtures, including gasoline. Current EPA regulations[11] stipulate that all gasoline imported and refined for sale in the United States contain benzene at ≤ 0.62 vol% on an annual average basis and a maximum content of 1.3 vol%. Historically, maximum concentrations close to 9% have been reported in European gasoline with mean values of 1–4%,[12] while US and North American gasoline has contained approximately 1–2%.

Despite these more recent reductions, benzene does remain a constituent in gasoline, hence the potential for dermal absorption remains. Apparently only two studies have measured *in vitro* benzene permeation from gasoline. Blank and McAuliffe[6] studied benzene penetration through heat-separated human epidermis from various vehicles including gasoline. Their formulation contained 5 vol% benzene (= 50 μl/ml = 43.7 mg/ml; density of benzene = 874 μg/μl), and the authors reported a J_{ss} of 0.07 ± 0.03 μl/cm^2/h (= 61 ± 26 μg/cm^2/h). More recently, Adami et al.[13] measured the *in vitro* penetration of benzene, toluene and xylenes from gasoline through full-thickness human abdominal skin. Benzene concentrations in the three tested gasoline formulations ranged from 0.39 to 1.06 wt%, and their measured J_{ss}s ranged from 1.47 ± 0.53 to 2.71 ± 1.62 μg/cm^2/h. Therefore, the flux measurement of Blank and McAuliffe is ~ 30-fold higher than the values by Adami et al., even though benzene concentration was only ~ 5-fold higher. This discrepancy has fueled debate over the assignment of appropriate flux values for benzene from gasoline.[4,14–16]

Additional data from a single laboratory using human skin processed in the same manner are necessary to fill in the gaps of the existing data and to cover a broader range of benzene concentrations that have been used historically. A well-characterized gasoline formulation containing 0.8 vol% benzene ("native gasoline") was used in these *in vitro* studies. Epidermal permeation experiments used this formulation alone and spiked with additional benzene up to 10 vol%, as well as neat benzene to determine steady-state fluxes and lag

times. To address differences in previously published permeation values, the penetration rates of benzene from native gasoline were compared between epidermis and full-thickness skin samples. Additionally, we measured the absorption kinetics of benzene from small (finite) gasoline doses of 10 $\mu l/cm^2$ containing 1.8 vol% benzene administered multiple times over a two-day period. Finally, we measured the thermodynamic activity of benzene in gasoline up to 100% (neat benzene). These new data enable more confident interpolation of flux data over the range of benzene concentrations that have been used historically in gasoline. This in turn should enhance confidence in both current-use exposure estimates and historical benzene exposure reconstructions. Implications of these data within dermal exposure assessments are provided with several examples. A subsequent report will present permeation results on toluene, ethylbenzene, xylenes and naphthalene from gasoline.

MATERIALS AND METHODS

Chemicals and Solutions

Gasoline reference material (“native gasoline”), supplied in 20 ml sealed glass ampules, was purchased from Supelco (Cat. no. 502227; lot LC03300) and stored at 4 °C. The accompanying Consensus Analysis reported a benzene concentration of 0.80 vol% (average of three determinations using ASTM D3606, D5580 and D5769). An ampule was submitted for further characterization to Bureau Veritas North America for analyses by ASTM D5580 and ASTM D6730 at their Houston Technical Center. Results (Table 1) confirmed a benzene concentration of 0.80 vol% (mean of both methods). With a density of 874 mg/ml, the benzene concentration in this gasoline is 6.99 mg/ml. Gasoline was transferred to minimal headspace Teflon-sealed glass vials, stored at 4 °C, and used within 2 weeks of opening. Benzene was from Sigma-Aldrich (Cat. no. 270709, lot SHBF0424V) with reported purity > 99.9%.

Receptor fluid and buffer were HEPES-buffered Hanks Balanced Salt Solution (Gibco, Invitrogen, Carlsbad, California, USA), with 50 mg/l of gentamicin sulfate and 5.96 g/l of HEPES (Sigma-Aldrich). The pH of the buffer was adjusted to 7.4 at 37 °C. The buffer was filtered (pore sizes: 0.2 μm, SFCA, Nalgene, Thermo Fisher Scientific) and degassed prior to use by warming to 40 °C and stirring under laboratory vacuum.

Human Skin Preparation

Human skin was received fresh from the West Virginia University Tissue Bank and was processed within 1 day of receipt. The surgical specimens were taken following informed consent from eight white females, ages 23–53 years, who had undergone breast reduction surgery. Our use of human tissue was deemed “not human research” by WVU and NIOSH Human Subject Review Boards. Heat-separated epidermal membranes (HEM) were prepared by submersing the skin in buffer at 60 °C for 60 s. Epidermis was separated from remaining dermis using cotton swabs. Epidermal discs were cut using a stainless steel punch (1.59 cm diameter), floated onto a pool of buffer with 10% glycerol on aluminum foil, covered with gauze and stored at − 85 °C. Full-thickness skin was prepared by scraping off subcutaneous fat prior to storage as with epidermis. Neither epidermal nor full-thickness

skin thicknesses were measured. Skin samples were stored up to 1 year prior to use. We have demonstrated retention of barrier properties under these conditions.[17]

In Vitro Permeation Studies

Franz-type (static) diffusion cells were used (0.64 cm^2 diffusion area and 5 ml receptor volume, PermeGear, Hellertown, PA, USA). Studies were conducted in a fume hood with certified face velocity between 56 and 70 cm/s and conformed to OECD Guidelines[7] except where noted. To minimize evaporation of benzene and other volatiles, custom (PermeGear) threaded glass donor cells were used and sealed with Teflon-septa caps. Sample side arms were also sealed with Teflon septa. Skin samples were thawed at room temperature, floated on a pool of buffer and mounted on cells with dialysis tubing (MWCO 12–14,000) used as a membrane support for HEM. Skin surfaces were rinsed 3 × with water and equilibrated overnight with buffer in receptor compartments. The skin surface was open to air and maintained at 32 °C by recirculating water at 37 °C through the jacketed cells. Relative humidity in the laboratory was not monitored. Studies were performed on 12 skin replicates for each formulation. Owing to limited sizes of the surgical specimens, not all experiments could be performed from the same human donors. Experiments using gasoline and gasoline fortified with up to 5% total benzene were performed on three samples from each of the same four individuals. Experiments using gasoline with 10% benzene and neat benzene shared three of these four individuals plus one additional. Studies comparing full thickness with epidermis were carried out on samples from one individual. Finite dose experiments were undertaken using four skin discs from each of the three individual donors who differed from those used in the infinite dose studies.

Receptor compartment samples were 0.5 ml and were replaced with fresh buffer. The samples were removed with a gas tight Hamilton syringe, placed in precooled 10 ml headspace vials, immediately capped and stored on a cooled (4 °C) sample tray until analyzed, within 38 h. Tests showed no significant loss of benzene from samples stored up to 48 h prior to analysis ($P > 0.9$, Pearson Product Moment Correlation).

Donor Formulations

Donor formulations consisted of native gasoline, gasoline+added benzene and neat benzene. None were radiolabeled. Working dilutions of gasoline+benzene were mixed the day of the experiment. Benzene was added to gasoline to form total concentrations (vol%) of 2.2, 3.6, 5.0 and 10. For finite dose studies, benzene was added to facilitate quantification for a total concentration of 1.8 vol%.

Infinite Dose Studies

Infinite dose studies permit calculation of steady-state fluxes, permeability coefficients and lag times. One thousand microliters of the donor formulation were applied to skin surface and donor cells were immediately capped (occluded condition) to inhibit evaporation of the volatile components with the goal to maintain a constant benzene concentration. The exposure duration for gasoline and gasoline+benzene formulations was 8 h, and receptor fluid was sampled at 0, 0.5, 1, 2, 3, 4, 6 and 8 h. For neat benzene, exposure duration was 4

h to ensure that benzene concentrations in receptor fluid remained approximately < 10% of aqueous solubility.

Finite Dose Studies

Finite dose studies are more representative of common gasoline-related exposure scenarios. Doses of 10 μl/cm^2 were used, in accordance with OECD guidelines.[7] The formulation was gasoline with 1.8 vol% benzene (benzene dose 157 μg/cm^2). Standard donor cells (internal diameter 0.9 cm; height 1.7 cm) were used and were not capped (unoccluded condition) to allow for volatile evaporation, which more closely reflects typical exposures. Receptor samples were taken at 0, 20, 40, 60 and 120 min, at which point an additional dose was added to donor cells, samples were taken and at 240 min, a third dose was added for a total exposure sample time of 360 min. The following day, at approximately the same time, the three doses were repeated for a total of six exposures. Benzene recovery, recommended in the OECD guideline,[7] was not determined. To do so would have required the placement of a trap above the donor cells that would have lowered the benzene evaporation rate compared with that from an open donor cell, with the expected consequence of greater benzene absorption.

Benzene Thermodynamic Activity Measurements

The thermodynamic activity of benzene (a_{Ben}) in gasoline solution was determined by measuring its equilibrium partial pressure relative to that of pure benzene measured at the same defined temperature.[18,19] Partial pressures were measured at 30 °C using static headspace gas chromatography (GC) described below.

Gasoline–benzene solutions were mixed to achieve a specified volume fraction ϕ_{Ben} (volume of benzene divided by total solution volume):

$$\phi_{\mathrm{Ben}} = \frac{V_{\mathrm{Ben}} + 0.008V_{\mathrm{Gas}}}{V_{\mathrm{Ben}} + V_{\mathrm{Gas}}}, \quad (1)$$

where V_{Ben} is the volume of added benzene and V_{Gas} the volume of gasoline. Results are presented as functions of both volume fraction and mole fraction x_{Ben}, which was calculated as:

$$x_{\mathrm{Ben}} = \frac{n_{\mathrm{Ben}}}{n_{\mathrm{Ben}} + n_{\mathrm{Gas}}} = \frac{\left(V_{\mathrm{Ben}} + 0.008\mathrm{V}_{\mathrm{Gas}}\right)\rho_{\mathrm{Ben}}/\mathrm{MW}_{\mathrm{Ben}}}{V_{\mathrm{Ben}}\rho_{\mathrm{Ben}}/\mathrm{MW}_{Ben} + V_{\mathrm{Gas}}\rho_{\mathrm{Gas}}/\mathrm{MW}_{\mathrm{Gas}}}, \quad (2)$$

where n is the number of moles, ρ is the density (g/ml) and MW is the molecular weight (g/mole). ρ_{Ben} is 0.874; $\mathrm{MW}_{\mathrm{Ben}}$ is 78.1; ρ_{Gas}, measured by us at uncontrolled room temperature (~22 °C) is 0.733 and $\mathrm{MW}_{\mathrm{Gas}}$ was taken as an “average value” of 108.[20] A total of three independent measurements were taken at each ϕ_{Ben}, except that nine measurements were made at $\phi_{\mathrm{Ben}} = 1$.

GC Analysis

Static headspace GC analysis was performed on samples from infinite dose studies and thermodynamic activity determinations. The GC system was a Varian 3800 with CombiPal autosampler using headspace mode. A flame ionization detector was used and the column was a trifluoropropyl-methyl polysiloxane capillary (Restek RTX 200 MS, 30 m length, 0.25 mm ID, 1 μm thickness) with 1 ml/min of He gas flow. Receptor cell samples were incubated at 50 °C for 20 min. Six hundred microliters of headspace was injected with split ratio at 10. The oven temperature was isothermal at 60 ° C and benzene retention time was 5.7 min. Calibrations were linear ($R^2 > 0.99$) over the range of sample values.

For activity measurements, samples were incubated at 30 °C for 30 min prior to injection with a split ratio of 50. Activity was calculated as the peak area of the sample, divided by the average peak area of the samples of neat benzene.

For finite dose benzene detection, the CombiPal ITEX preparation method of sample enrichment was used that repeatedly (20 times) draws a 1000 μl headspace sample through a Tenax-coated needle prior to rapid thermal desorption (230 °C) into the GC injector. Highly linear ($R^2 > 0.99$) calibrations were obtained with benzene concentrations down to 3 ng/ml.

Calculation of Permeation Parameters

Steady-state fluxes (J_{SS}), permeability coefficients (k_p) and lag times (t_{lag}) were calculated from infinite dose experimental results. The total amount of chemical that penetrated the skin and was absorbed into the receptor fluid, normalized by the area of exposed skin ($m(t)$), was calculated from the measured receptor concentrations at each sample time point, taking into account the amount that had been removed with each prior sample. J_{ss} and t_{lag} were calculated by nonlinear regression of the experimental data to the solution of the one-dimensional diffusion equation for a homogeneous membrane initially free of chemical and exposed on the outer surface to a constant concentration while sink conditions (zero concentration) apply to the inner surface. For each dosed skin sample, the best fit of the data to the first seven terms of the solution[21] was determined using SigmaPlot 12.5 (Systat Software):

$$m(t) = J_{SS}t - J_{SS}t_{lag} - 12\frac{J_{SS}t_{lag}}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(-n^2\pi^2\frac{t}{6t_{lag}}\right) \quad (3)$$

The analysis yields estimates for the two variables J_{ss} and t_{lag}. The permeability coefficient, k_p, was calculated as

$$k_p = \frac{J_{SS}}{C_d}, \quad (4)$$

where C_d is the (constant) concentration of benzene in the donor formulation. For neat benzene, the concentration was taken as its density (874 mg/ml).

Fractional absorption of benzene from finite dose exposures was calculated as the total mass recovered in receptor cells at 2 h of exposure, divided by the applied benzene dose (both: mass/area).

Statistical Tests

The number of replicates for these studies was chosen to exceed OECD guidelines.[7] Preexposure assessment of barrier function was not undertaken. All replicates that were mounted on diffusion cells were dosed and all data were included in subsequent analyses and statistical tests: no replicates were rejected by *post hoc* evaluation. Samples were not allocated randomly, but replicates from each human donor were chosen haphazardly for treatment. The investigator was not blinded either during the experiment or when analyzing data. In contrast with commonly used graphical methods to determine steady-state fluxes and lag times, the use of Eq. (3) removes subjectivity from this analysis. Statistical tests were conducted using SigmaPlot 12.5. Differences between- or among-group means were probed using unpaired *t*-test, one-way analysis of variance or repeated-measures one-way analysis of variance. If normality test (Shapiro–Wilk) failed, then a non-parametric analysis (Mann–Whitney rank-sum test, Kruskal–Wallis one-way analysis of variance on ranks or Friedman repeated-measures analysis of variance on ranks) was performed. Specific tests are indicated in the Results section. Significance was accepted where $P < 0.05$.

RESULTS

Infinite Dose Studies

Figure 1 displays benzene permeation curves from all infinite dose epidermal exposures. These are pooled data displaying means and SDs of all 12 samples for each formulation. Solid lines represent the regression of the data with Eq. (3). These data show conformity with the characteristics of an archetypal infinite dose exposure: after a measurable lag time, a steady-state flux rate, characterized by the linear portion of the curve, is approached. Such linearity is achieved if a relatively constant concentration difference is maintained between donor and receptor.

Table 2 presents results from infinite dose epidermal permeation studies for benzene concentrations (vol%) of 0.8, 2.2, 3.6, 5.0, 10 and 100. Steady-state fluxes, permeability coefficients and lag times are listed. Correlations with Eq. (3) for all formulations were excellent ($R^2 > 0.99$) and variances (< 32%) were reasonable for steady-state flux measurements. Permeability coefficients for formulations up to 10% benzene showed no significant differences (Kruskal–Wallis one-way analysis of variance on ranks). This finding indicates that steady-state flux is a near linear function of benzene concentration over this range.

For lag times, variances as high as 132% were observed and showed no significant differences for formulations up to 10% benzene (Kruskal–Wallis one-way analysis of variance on ranks). It appeared from an examination of the data in Figure 1 that a minor but noticeable falloff in benzene mass after 4 h may have contributed to a small lag time estimate with large variance. This observation led to a reanalysis of data considering only

the first 4 h of gasoline exposure. The calculations for J_{SS}, k_p and t_{lag} from this analysis are shown in Table 2 in parentheses following the full 8 h data. These estimates of t_{lag} ranged from 0.25 to 0.29 h with smaller variances (41–52%) ($P < 0.05$ compared with 8 h estimates for all except 2.2 vol%). On the other hand, estimates of J_{SS} (and k_p) from the 4 h exposure data were minimally (< 10%) and insignificantly greater than the 8 h estimates.

Figure 2 displays benzene permeation curves comparing human epidermis and full-thickness skin samples exposed to native gasoline (0.8 vol%). Again, pooled data are shown as means and SDs and solid lines represent regressions of the data with Eq. (3). These data also show excellent conformity with the permeation characteristics of an infinite dose exposure: correlations with Eq. (3) were excellent ($R^2 > 0.99$).

Table 3 presents results comparing epidermis and full-thickness skin samples. Although these studies were derived from samples from a single human donor, the epidermal flux values did not differ significantly from the values reported in Table 2 for the same formulation, indicating that these skin samples are representative. The J_{SS} (and k_p) of epidermis are ~ 4.5-fold greater than those of the full-thickness specimens, while the lag time of the full-thickness skin was nearly 9-fold greater than the epidermis lag time. Both J_{SS} and lag time measurement differences between the two skin preparations were highly significant ($P < 0.001$, two-tailed *t*-test).

Finite Dose Studies

Figure 3 displays pooled benzene permeation curves following three successive finite doses of 10 μl/cm^2 of gasoline with 1.8 vol% benzene. Each dose therefore equals 157 μg benzene per cm^2 exposure. Doses were administered 120 min apart; note that benzene penetration had leveled off at that time, indicative of near completion of the kinetic processes of absorption/evaporation. Solid lines connect the data as a guide to the eye.

Figure 4 shows box plots and individual ($n = 12$) scatter plots of benzene penetration for all six finite dose exposures over a two-day period. Benzene penetration following each dose was calculated as the amount at 120 min post exposure, minus the amount immediately preceding the exposure. Results are presented as total area-normalized benzene mass and as percentage of absorption of the applied benzene dose. Doses 5 and 6 exhibited significant differences compared with Dose 1 ($P < 0.05$, Friedman repeated-measures analysis of variance on ranks; multiple comparisons *versus* Dose 1 using Dunnett's method). Also, the median of all day-1 exposures (0.104 μg/cm^2) was significantly less than the median of all day-2 exposures (0.129 μg/cm^2) ($P < 0.05$, Mann–Whitney rank-sum test).

Thermodynamic Activity

Figure 5 displays measured thermodynamic activity of benzene in benzene–gasoline mixtures. Benzene was miscible in gasoline at all concentrations. Activities are displayed as functions of mole fraction (A) and volume fraction (B) of benzene. Solid lines represent ideal solution behavior.

DISCUSSION

The data described herein complement and extend previous studies on benzene permeation from gasoline and provide a reasonable explanation for apparent discrepancies from those studies.

Infinite Dose Studies

The soundness of the steady-state flux measurements reported here are supported by excellent correlations with Eq. (3) for all formulations ($R^2 > 0.99$) and by variances < 32%.

Gasoline donor solution fluxes.—If steady-state flux is a linear function of benzene concentration, then the permeability coefficient of benzene in gasoline will be a constant (Eq. (4)). Our data show that this is the case for benzene concentrations up to 10% (Table 2): no significant differences were observed (Analysis of Variance) among k_ps in this concentration range. This is complemented by the finding that benzene–gasoline mixtures appear to form a nearly ideal solution over the entire concentration range (Figure 5): the magnitude of thermodynamic activity nearly equals the benzene mole fraction. For a fully miscible solution, activity is related to mole fraction as:

$$a_{\text{Ben}} = Y_{\text{Ben}} X_{\text{Ben}}, \quad (5)$$

where γ_{Ben} is the activity coefficient. Thus, for an ideal solution, $\gamma_{\text{Ben}} = 1$. When plotted as volume fraction (Figure 5b), curvature is observed (owing to differences in the ratios of density to MW of the two substances; see Eq. (2)) but for volume fractions up to at least 0.2, a linear approximation seems warranted. Therefore, a near linear relationship between steady-state flux and benzene concentration should be expected within this range. Our data support this expectation for concentrations up to 10 vol% (Figure 6).

Neat benzene fluxes.—However, the steady-state flux of neat benzene is less than what would be expected from linear extrapolation from the measured quantities up to 10% benzene. Based on results in Table 2, a J_{ss} of ~ 1000 μg/cm^2/h would be expected, while 566 μg/cm^2/h was measured. As a solvent, one might expect that neat benzene may reduce barrier function by solvating stratum corneum (SC) lipids. This appears not to be the case here and the reason for a lower than expected neat flux is unknown. The 16-h neat benzene data by Lodén[5] show no sign of barrier reduction over a 16-h exposure, as evidenced by excellent linearity of the mass absorption profile, and Blank and McAuliffe[6] also report no indication of barrier disruption to benzene flux.

Others have reported in vitro human neat benzene fluxes. The reported mean value of 99 μg/cm^2/h by Lodén[5] was obtained using full-thickness skin which, as discussed subsequently, presents an un-physiologically high aqueous diffusion barrier that impedes transdermal flux. If one assigns the same factor of difference (4.5-fold) reported here for benzene flux from gasoline mixtures between epidermis and full-thickness skin, an estimated epidermal J_{SS} of 447 μg/cm^2/h results.

The highest published human neat benzene mean flux value is of 2.11 μl/cm^2/h by Blank and McAuliffe[6] or 1844 μg/cm^2/h. Others[4,9,10] have questioned this based on an analysis of a single experiment displayed in Figure 1 in the study by Blank and McAuliffe.[6] OSHA[9] estimated J_{SS} from this curve to be 560 μg/cm^2/h. We disagree with this claim of a "corrected" value and accept Blank and McAuliffe's reported value derived, as they claim, "from multiple experiments". However, do recognize the close similarity of the OSHA estimate with our measured mean of 566 μg/cm^2/h.

Franz[22,23] reported total mass absorbed from a range of neat loads applied to split-thickness skin. Only the two highest doses (270 and 520 μl/cm^2) may have reached a steady state within Franz's estimated exposure times of 1.5 and 3 h, respectively. Williams et al.[4] calculated fluxes of 260 and 330 μg/cm^2/h but did not account for lag times in their estimations. For a lag time of 0.6 h (Table 2), Franz's presumed steady-state fluxes (mass absorbed/(exposure time − lag time)) would be 329 and 544 μg/cm^2/h.

A classic study by Hanke et al.,[24] originally published in 1961, described human *in vivo* neat benzene exposures and reported a J_{SS} of 400 μg/cm^2/h, based on benzene loss over the exposure duration of 1.25–2 h. This flux calculation is thus time averaged and did not consider the contribution of a possible lag time. For a lag time of 0.6 h, this flux may be estimated (reported J_{SS} × exposure duration)/(exposure duration − lag time) as 769 or 571 μg/cm^2/h, respectively.

These reports are the only studies from which a neat steady-state human benzene flux has either been reported or can be calculated. The calculated fluxes based on the data by Franz[22,23] and Hanke et al.[24] depend, of course, on the specific value of lag time used: shorter lag times will result in lower flux values. In the following section, we argue that a lag time range of 0.3–0.6 h is reasonable for neat benzene.

Lag time estimates.—Calculated lag times for gasoline exposures exhibited high variances. A potential issue with data analysis was identified in the Results section, and reanalysis yielded lag times of ~ 0.25 h with substantially less variance. This exercise points out the sensitivity of lag time measurements to small trends in the absorption rate over time. We posit 0.25 h as a more accurate estimate of lag time for benzene–gasoline mixtures. Apparently no other reports of epidermal benzene lag times from gasoline exposures *in vitro* are available for comparison. For full-thickness skin, our measured value 2.20 ± 1.01 h is in broad agreement with the range of mean values from 0.75 to 1.54 h by Adami et al.[13] for three gasoline formulations.

The lag time of neat benzene measured here was 0.59 ± 0.15 h (Table 2). Although Blank and McAuliffe[6] did not report lag times, extrapolation of the single neat benzene exposure shown in their Figure 1 suggests a value of ~ 0.5 h. Franz[22,23] likewise did not report neat benzene lag times through their split-thickness skin, but analysis of the flux data in his Figure 3 suggests a value of ~ 0.3 h for pig skin. Franz reported that the times to maximum flux for human skin were ~ 2-fold larger than for pig skin, so a lag time of 0.5 h seems a reasonable estimate. We[25] previously reported a human epidermal lag time of 0.15 h for benzene-saturated aqueous donor. However, direct comparisons in our laboratory[26,27] of

permeation data for other compounds showed that neat donor lag times were ~ 1.5–3-fold longer than aqueous donor lag times (Frasch and Barbero[27] present a discussion on this point). Thus all relevant published data suggest a reasonable estimate of 0.3–0.6 h for lag time of neat benzene through epidermal or split-thickness human skin.

Examination of previously reported flux discrepancies.—Our data clarify discrepancies in previously published *in vitro* data on steady-state benzene flux from gasoline exposure. Figure 6 compares our measurements with data from Blank and McAuliffe[6] and from Adami et al.[13] Solid circles represent our data (mean, SD) on steady-state flux measurements with benzene concentration in gasoline, using human epidermis. The open circle is the reported value of 61.2 ± 25.5 μg/cm^2/h by Blank and McAuliffe also using human epidermis. The solid square represents our value for full-thickness skin, and open squares are the data reported from Adami et al., who used full-thickness skin. (Adami et al. report benzene concentrations as wt%, which have been transformed here to vol% by multiplying by the ratio of gasoline-to-benzene densities.) The inset to Figure 6 shows these full-thickness data more clearly.

The dashed line in Figure 6 is a plot of proposed relationship by Petty et al.[14] (their Eq. (11)) to predict steady-state benzene flux from concentration, based on regression of data from the literature:

$$J_{SS}\left(\mu g/\mathrm{cm}^2/h\right) = 20.4 \times (\text{benzene vol\%})^{0.6616}. \quad (6)$$

This relationship was proposed for the complete range of benzene concentrations up to 100% and for any formulation containing benzene. However, our epidermis data in Figure 6 demonstrate the superiority of a simple linear relationship (solid line) for gasoline with benzene concentrations up to 10 vol%. Based on these new data, we propose the following to be used to predict flux from gasoline with benzene concentrations that represent current and historical formulations:

$$J_{SS}\left(\mu g/\mathrm{cm}^2/h\right) = 1.4 + 10.6 \times (\text{benzene vol \%}), \quad (7)$$

which correlates with current measurements with an R^2 of 0.92. No data support the use of this relationship for > 10 vol% benzene.

As mentioned previously, discrepancies in measured benzene flux data from various organic mixtures has generated a heated debate surrounding the appropriate values to be used in estimating human dermal absorption for risk assessments. Data generated here should help to resolve the controversy for gasoline, as the discrepancies can be explained simply by considering the skin preparation technique. Flux measurements by Blank and McAuliffe[6] are in complete accord with our measured values using human epidermis, while those of Adami et al.[13] are in complete accord with our values using full-thickness human skin. The thick (~1–2 mm), watery dermis impedes the penetration of the moderately liphophilic benzene. *In vivo*, the dermis is vascularized and convection (blood flow) is the primary

transport mechanism of chemicals that penetrate the epidermis, where transport is by diffusion. The *in vitro* full-thickness preparation thus presents a thick aqueous barrier that is not present *in vivo*. The isolated epidermis with sink conditions at the inner surface is the more appropriate *in vitro* model for the *in vivo* setting. In cases where *in vitro* studies are used to support dermal risk assessments, we propose that data from full-thickness skin measurement should not be used barring a proper accounting of this aqueous barrier. The same argument would question neat benzene flux value of 99 $\mu g/cm^2/h$ by Lodén[5] as being artificially low.

Finite Dose Studies

The finite dose permeation data (Figures 3 and 4) show a fractional absorption of < 0.25% of the benzene from finite dose applications of 10 $\mu l/cm^2$ gasoline left open to air. To enhance accuracy of quantification, benzene was added to gasoline to a final concentration of 1.8 vol % for a benzene mass load of 157 $\mu g/cm^2$. Less than 0.4 $\mu g/cm^2$, with a median value of 0.11 $\mu g/cm^2$ (0.07% of the applied load) benzene was absorbed per exposure. To our knowledge, these are the only data that measure benzene permeation from finite dose gasoline exposures.

The total mass of chemical absorbed from finite doses depends on the rate of chemical evaporation compared with the rate of skin absorption.[28,29] For *in vitro* studies, the evaporation rate of volatiles from the skin surface will vary with the dimensions of the donor cells (height:diameter ratio) (Frasch, unpublished) as well as the ambient air velocity[30] (here, 56–70 cm/s).

The gasoline exposure experiments undertaken herein under our laboratory conditions are in good agreement with previous studies using neat benzene under a variety of conditions, both *in vitro* and *in vivo*. Maibach and Anjo[31] reported 0.17% absorption from ~ 4 $\mu l/cm^2$ neat benzene applied to rhesus monkeys *in vivo*; Modjtahedi and Maibach[32] observed absorptions of 0.07 and 0.13% from exposures of ~ 1 $\mu l/cm^2$ applied to human forearms and palms, respectively. Franz[22,23] reported 0.1 and 0.05% absorption of neat benzene in human *in vitro* and *in vivo* studies, respectively, while Gajjar and Kasting[33] measured ~ 0.05% *in vitro*.

The initial sample interval of 15 min in our experiments was too long to calculate the rapidly changing benzene absorption rate: Gajjar and Kasting[33] report maximum finite dose fluxes for neat benzene to occur within 5–20 min after exposure. For risk assessment, the total amount of chemical that is absorbed from a finite dose, rather than the non-steady absorption rate, is the crucial metric. Fitting of the data to an appropriate diffusion model[33] could be performed if time-dependent flux estimates are needed.

Our finite dosing experiments covered only 1 gasoline load of 10 $\mu l/cm^2$ and 1 benzene concentration. Different total mass absorptions would be expected from different loads. Gajjar and Kasting[33] observed this for neat benzene loading but their cumulative 24-h percentage of absorption did not vary much (0.04–0.05%) over an 8-fold dose range. Therefore, total mass absorption from finite dose gasoline exposures may reasonably be

predicted from the fractional absorption measured here multiplied by the applied benzene mass load.

From multiple exposures over 2 days (Figure 4), there is some evidence of diminished barrier function. Doses 5 and 6 exceed dose 1 ($P < 0.05$, one-way analysis of variance on repeated measures), and the median of day-2 exposures (0.129 μg/cm^2, combined doses 4–6) exceeded that of day-1 exposures (0.104 μg/cm^2, combined doses 1–3) ($P < 0.05$, Mann–Whitney rank-sum test). However, we cannot dismiss a nonspecific deterioration of the barrier over 2 days in an *in vitro* system.

Use of Data in Exposure Assessments

Results from these studies can be used to estimate benzene uptake from gasoline and neat benzene skin contact for exposure assessment purposes. The following are presented to demonstrate applications of data presented herein, and the authors make no claims regarding the realistic nature of these exposures.

Comparison with inhalation uptake rate.—These calculations are presented in order to compare the extent of dermal exposure that is necessary to equal the benzene uptake from inhalation. The OSHA benzene permissible exposure limit (PEL) for 8 h time-weighted average is 1 p.p.m., which equals 3.19 mg/m^3 air concentration at 25 °C and 760 mmHg. The EPA[34] recommends a breathing rate of 0.72 m^3/h for light activity among adults. A pulmonary uptake fraction of 0.5 leads to 1.15 mg/h total benzene uptake rate through inhalation. For gasoline with 2 vol% benzene (17.5 μg/μl), Eq. (7) predicts a dermal steady-state flux of 22.6 μg/cm^2/h, so that a fully immersed skin surface area of 51 cm^2 would result in equivalent steady-state systemic benzene uptake rate (3.19 mg/m^3 × 0.72 m^3/h × 0.5 = 1.15 mg/h = 22.6 μg/cm^2/h × 51 cm^2). This area represents a small (< 4%) fraction of the EPA estimation of total surface area of adult male hands (1310 cm^2).[34]

Transient immersion exposure.—Full-time immersion of skin in gasoline is an unrealistic exposure. Here we assume a 2 min immersion of both hands in gasoline with 2 vol% benzene. Previous analyses[35,36] have provided the theoretical underpinnings for the following equation that is commonly used in dermal risk assessments. Equation (8) quantifies total mass uptake (m_T) from a transient immersion exposure to a highly volatile chemical, even if the exposure duration is significantly less than the membrane lag time for the chemical:

$$m_T = J_{SS} A_{exp} t_{exp}, \quad (8)$$

where A_{exp} and t_{exp} are exposed skin area and exposure duration. The assumption here is that the skin is thoroughly decontaminated following t_{exp}. For a 2-min exposure to the hands, this leads to an estimated systemic benzene uptake of about 1 mg of benzene (22.6 μg/cm^2/h × 1310 cm^2 × 2/60 h = 987 μg). If we assume that a film of 10 μl/cm^2 of gasoline remains on skin after the 2-min exposure until evaporation, we can use the finite dose results with benzene absorption of 0.25% of applied dose to predict additional uptake of 573 μg (10

$\mu l/cm^2 \times 1310\ cm^2 \times 17.5\ \mu g/\mu l \times 0.25\%/100\% = 573\ \mu g$). This total benzene uptake of 1.56 mg corresponds to that from ~ 1.4 h of inhalation uptake at 1 p.p.m.. Petty et al.[14] estimate benzene uptake of 3.07 mg for a similar dermal exposure. The main difference is their calculated steady-state flux, based on Eq. (6), of 32.3 $\mu g/cm^2/h$.

Another way of considering immersion exposures follows. Over the course of an 8-h day, any product of exposure surface and exposure duration equal to 407 $cm^2 \cdot h$ would be equivalent to the OSHA PEL pulmonary uptake of 9.2 mg (22.6 $\mu g/cm^2/h \times 407\ cm^2 \cdot h = 9.2$ mg = 3.19 $mg/m^3 \times 0.72\ m^3/h \times 8\ h \times 0.5$). For example, full immersion of both hands for 0.31 h (~19 min) would be equivalent. This assumes thorough skin surface decontamination following the exposure; in the absence of which, dermal exposure will contribute more than respiratory.

Splash exposures.—Finite dose exposures are highly relevant to typical occupational and consumer scenarios and may contribute substantially to overall body burden.[37] Skin exposures to small splashes of chemical, including gasoline, can go unnoticed,[38] which highlights the importance of recognizing and quantifying these exposures. Because of the rapid evaporation rate of benzene, the total mass uptake from a splash or non-occluded finite dose exposure is expected to be small. If we assume 1 drop equals 50 μl, and each drop covers 5 cm^2 of skin (10 $\mu l/cm^2$), then, based on the finite dose results of 0.25% benzene absorption, total benzene uptake from 2 vol% gasoline (17.5 $\mu g/\mu l$) corresponds to 2.19 μg per drop (50 $\mu l \times 17.5\ \mu g/\mu l \times 0.25\%/100\% = 2.19\ \mu g$). Therefore, 526 drops per hour onto skin would be equivalent to the inhalation uptake at 1 p.p.m.

Galea et al.[38] presented data on consumer exposures to diesel fuel from vehicle tank filling. Total exposure to both hands and forearms at the 90th percentile was 42.8 μg of fuel per cm^2 of exposure. The authors report total exposed skin area to be 990 cm^2 for males. We assume 2 vol% benzene (0.02 ml/ml) in gasoline and absorption of 0.25% of the exposure. For precise calculation, vol% is transformed to mass%: 0.02 ml benzene/ml gasoline × 874 μg benzene/μl benzene÷733 μg gasoline/μl gasoline = 0.024 μg benzene/μg gasoline. Total systemic benzene uptake would therefore be 2.54 μg (42.8 μg gasoline/$cm^2 \times 990\ cm^2 \times 0.024\ \mu g$ benzene/μg gasoline × (0.25/100)% = 2.54 μg benzene). This corresponds to the inhalational uptake from 1 p.p.m. benzene vapor for ~ 8 s (1.15 mg/h × 1 h/60 min × 1 min/60 s × 7.95s = 2.54 μg).

Summary and Limitations

The present studies were designed to expand upon and interpret previous measurements of benzene permeation from gasoline and neat benzene. Using HEM, we found steady-state flux rates to be a linear function of the concentration of benzene in gasoline from 0.8 to 10 vol%. This linearity is expected based on our finding that benzene-gasoline mixtures behave as a near-ideal solution over the entire range of benzene concentrations. Steady-state fluxes through full-thickness skin from native gasoline containing 0.8 vol% benzene were ~ 4.5-fold less than the fluxes through epidermis. Measured steady-state fluxes at 0.8 and 5 vol% were similar to those reported by others using full-thickness (Adami et al) and epidermis (Blank and McAuliffe) membranes, respectively. Finite dose exposures to repeated single

volumetric loads of 10 μl/cm^2 of gasoline, corresponding to benzene mass loads of 157 μg/cm^2, demonstrated that < 0.25% of the total applied benzene dose was absorbed 2 h after the exposures. A median value of ~ 0.07% is in line with neat benzene data reported by others, both *in vitro* and *in vivo*. Applications of the data presented here in dermal exposure assessments is presented through several examples relevant to both occupational and consumer exposures.

These data are *in vitro* and so caution applies to their application to the *in vivo* setting. The experiments comparing full thickness with epidermis used skin samples (n = 12 each) derived from only one human donor and may not be representative of others. The finite dose experiments tested only one loading of one concentration; a broader range of studies would be desirable. Meaningful non-steady-state flux measurements would be possible with shorter sampling intervals during the kinetic phase. Only absorbed amounts were quantified and we did not measure evaporated mass from the diffusion cell apparatus or mass of benzene remaining in the skin following exposures. Therefore, a full accounting for disposition of the applied loads could not be made.

CONCLUSIONS

The data presented here compliment and extend previous studies on benzene dermal uptake from gasoline and neat benzene exposures. These studies provide a reasonable explanation of previously published conflicting benzene steady-state flux values and provide a framework for dermal exposure assessments to gasoline from current and historic gasoline formulations. Finite dose gasoline exposure results are presented for the first time, and repeat dosing suggests only minor alterations of the skin barrier under the conditions tested here.

These results may be used to estimate the extent to which benzene uptake can be expected from dermal exposures. On an area-normalized basis, extended immersion-type exposures lead to the greatest uptake, short-term exposures lead to less uptake and splash-type exposures are expected to contribute to the least uptake, owing to the volatility of benzene.

ACKNOWLEDGEMENTS

Intramural funding for this research was provided by the National Institute for Occupational Safety and Health, a part of the Centers for Disease Control and Prevention. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

REFERENCES

1. Benzene (CASRN 71-43-2). Integrated Risk Information System (IRIS), United States Environmental Protection Agency 2003: available at http://www.epa.gov/iris/subst/0276.htm.
2. Benzene. Report on Carcinogens, 12th edn National Toxicology Program, National Institute for Environmental Health and Safety 2011: available at http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/benzene.pdf.
3. Benzene. IARC Monographs on the Evaluation of Carcinogenic Risks in Humans Vol. 100F World Health Organization 2012: available at http://monographs.iarc.fr/ENG/Monographs/vol100F/mono100F-24.pdf.

4. Williams PRD, Sahmel J, Knutsen J, Spencer J, Bunge AL. Dermal absorption of benzene in occupational settings: estimating flux and applications for risk assessment. Crit Rev Toxicol 2011; 41: 111–142. [PubMed: 21288163]
5. Lodén M The in vitro permeability of human skin to benzene, ethylene glycol, formaldehyde, and n-hexane. Acta Pharmacol Toxicol 1986; 58: 382–3899.
6. Blank IH, McAuliffe DJ. Penetration of benzene through human skin. J Invest Dermatol 1985; 85: 522–526. [PubMed: 4067326]
7. OECD guideline for the testing of chemicals # 428. Skin absorption: in vitro methods, Organization for Economic Cooperation and Development: Paris 2004: available at http://www.oecd-ilibrary.org/environment/test-no-428-skin-absorption-in-vitro-method_9789264071087-en.
8. Bromhead, J; Permeation of benzene, trichloroethene and tetrachloroethene through plastic pipes: an assessment for Drinking Water Inspectorate. 1997. Report published online http://dwi.defra.gov.uk/research/completed-research/reports/dwi0772.pdf.
9. Occupational Exposure to Benzene; Final Rule. 29 CFR Part 1910. Federal Register Vol. 52, No. 176, 11 9 1987, p. 34489.
10. Nies E, Korinth G. Commentary on “Penetration of benzene, toluene and xylenes contained in gasolines through human abdominal skin in vitro”. Toxicol In Vitro 2008; 21: 275–277.
11. Control of Hazardous Air Pollutants From Mobile Sources; Final Rule. 40 CFR Parts 59, 80, 85, and 86. Federal Register Vol. 72, No. 37, 26 2 2007, pp. 8428–8570.
12. Verma DK, des Tombe K. Benzene in gasoline and crude oil: occupational and environmental implications. AIHA J 2002; 63: 225–230.
13. Adami G, Larese F, Venier M, Barbieri P, Lo Coco F, Reisenhover E. Penetration of benzene, toluene and xylenes contained in gasolines through human abdominal skin *in vitro*. Toxicol In Vitro 2006; 20: 1321–1330. [PubMed: 16829017]
14. Petty SE, Nicas M, Boiarski AA. A quantitative method for estimating dermal benzene absorption from benzene-containing hydrocarbon liquids. Int J Occup Environ Health 2011; 17: 287–300. [PubMed: 22069926]
15. Williams PRD, Sahmel J, Bunge AL, Knutsen J, Spencer J. Comments on Petty et al. (2011), “A quantitative method for estimating dermal benzene absorption from benzene-containing hydrocarbon liquids,” IJOEH 17: 287–300. Int J Occup Environ Health 2013; 19: 147–154. [PubMed: 23866376]
16. Petty SE, Nicas M, Boiarski AA. Authors’ response to Comments on Petty et al. (2011), “A quantitative method for estimating dermal benzene absorption from benzene-containing hydrocarbon liquids,” IJOEH, 17: 287–300 by Pamela R.D. Williams, Jennifer Sahmel, Annette L. Bunge, Jeffrey Knutsen, and John Spencer. Int J Occup Environ Health 2013; 19: 147–154. [PubMed: 23866376]
17. Barbero AM, Frasch HF. Effect of frozen human epidermis storage duration and cryoprotectant on barrier function using two model compounds. Skin Pharmacol Physiol 2016; 29: 31–40. [PubMed: 26606593]
18. Al-Khamis K, Davis SS, Hadgraft J, Mill S. The determination of thermodynamic activity by gas chromatography head space analysis and its use in studying release rate of drugs from topical preparations. Int J Pharm 1982; 10: 25–28.
19. Frasch HF, Barbero AM, Dotson GS, Bunge AL. Dermal permeation of 2-hydroxypropyl acrylate, a model water-miscible compound: effects of concentration, thermodynamic activity and skin hydration. Int J Pharm 2014; 1460: 240–247.
20. Toxicological profile for automotive gasoline. Agency for Toxic Substances and Disease Registry 1995: http://www.atsdr.cdc.gov/toxprofiles/TP.asp?id=468&tid=83.
21. Crank J The Mathematics of Diffusion, 2nd edn Clarendon Press: Oxford, UK, 1975 pp 51.
22. Franz TJ. Percutaneous absorption of benzene In: McFarland HN (ed). Proceedings of the Symposium: The Toxicology of Petroleum Hydrocarbons. American Petroleum Institute: Washington DC, USA, 1982 pp 108–114.
23. Franz TJ. Percutaneous absorption of benzene In: Applied Toxicology of Petroleum Hydrocarbons (Advances in Modern Environmental Toxicology, vol. 6) MacFarland HN (ed). Princeton Scientific Publishers: Princeton, USA, 1984 pp 61–70.

24. Hanke J, Dutkiewicz T, Piotrowski J. The absorption of benzene through the skin (translation from Polish). Medcyna Pracy 1961; 12: 413–426. English translation published in Int J Occup Environ Health 2000; 6: 104–111.
25. Frasch HF, Barbero AM. A paired comparison between human skin and hairless guinea pig skin in vitro permeability and lag time measurements for 6 industrial chemicals. Cutan Ocul Toxicol 2009; 28: 107–113. [PubMed: 19552540]
26. Frasch HF, Barbero AM, Dotson GS, Bunge AL. Dermal permeation of 2-hydroxypropyl acrylate, a model water-miscible compound: effects of concentration, thermodynamic activity and skin hydration. Int J Pharm 2014; 460: 240–247. [PubMed: 24239832]
27. Frasch HF, Barbero AM. In vitro human epidermal permeation of nicotine from electronic cigarette refill liquids and implications for dermal exposure assessment. J Expo Sci Environ Epidemiol, advance online publication 7 December 2016; doi:10.1038/jes.2016.68.
28. Kasting GB, Miller MA. Kinetics of finite dose absorption through skin 2: volatile compounds. J Pharm Sci 2006; 95: 268–280. [PubMed: 16381026]
29. Frasch HF. Dermal absorption of finite doses of volatile compounds. J Pharm Sci 2012; 101: 216–219.
30. Gajjar RM, Miller MA, Kasting GB. Evaporation of volatile organic compounds from human skin in vitro. Ann Occup Hyg 2013; 57: 853–865. [PubMed: 23609116]
31. Maibach HI, Anjo DM. Percutaneous penetration of benzene and benzene contained in solvents used in the rubber industry. Arch Environ Health 1981; 36: 256–260. [PubMed: 7294890]
32. Modjtahedia BS, Maibach HI. In vivo percutaneous absorption of benzene in man: forearm and palm. Food Chem Toxicol 1997; 46: 1171–1174.
33. Gajjar RM, Kasting GB. Evaporation of ethanol, acetone, benzene and 1,2-dichloroethane through human skin in vitro: a test of diffusion model predictions. Toxicol Appl Pharmacol 2014; 281: 109–117. [PubMed: 25283951]
34. US EPA. Exposure Factors Handbook: 2011 Edition. National Center for Environmental Assessment: Washington, DC, USA, EPA/600/R-09/052F.
35. Frasch HF, Barbero AM. The transient dermal exposure: theory and experimental examples using skin and silicone membranes. J Pharm Sci 2008; 97: 1578–1592. [PubMed: 17722104]
36. Frasch HF, Bunge AL. The transient dermal exposure II: post-exposure absorption and evaporation of volatile compounds. J Pharm Sci 2015; 104: 1499–1507. [PubMed: 25611182]
37. Frasch HF, Dotson GS, Bunge AL, Chen C-P, Cherrie JW, Kasting GB et al. Analysis of finite dose dermal absorption data: implications for dermal exposure assessment. J Expo Sci Environ Epidemiol 2014; 24: 65–73. [PubMed: 23715085]
38. Galea KS, Davis A, Todd D, MacCalman L, McGonagle C, Cherrie JW. Dermal exposure from transfer of lubricants and fuels by consumers. J Expo Sci Environ Epidemiol 2014; 24: 665–672. [PubMed: 24938510]

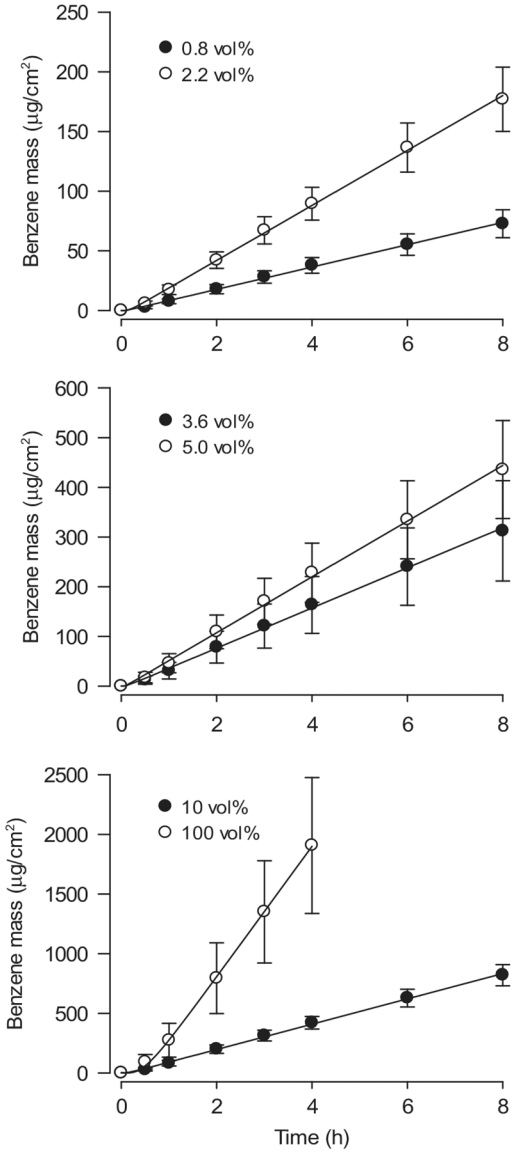

Figure 1.

Pooled benzene epidermal permeation curves (mean ± SD, $n = 12$ each) from infinite doses of gasoline at the indicated benzene vol% (100 vol% is neat benzene). Solid lines are regressions of mean data with Eq. (3).

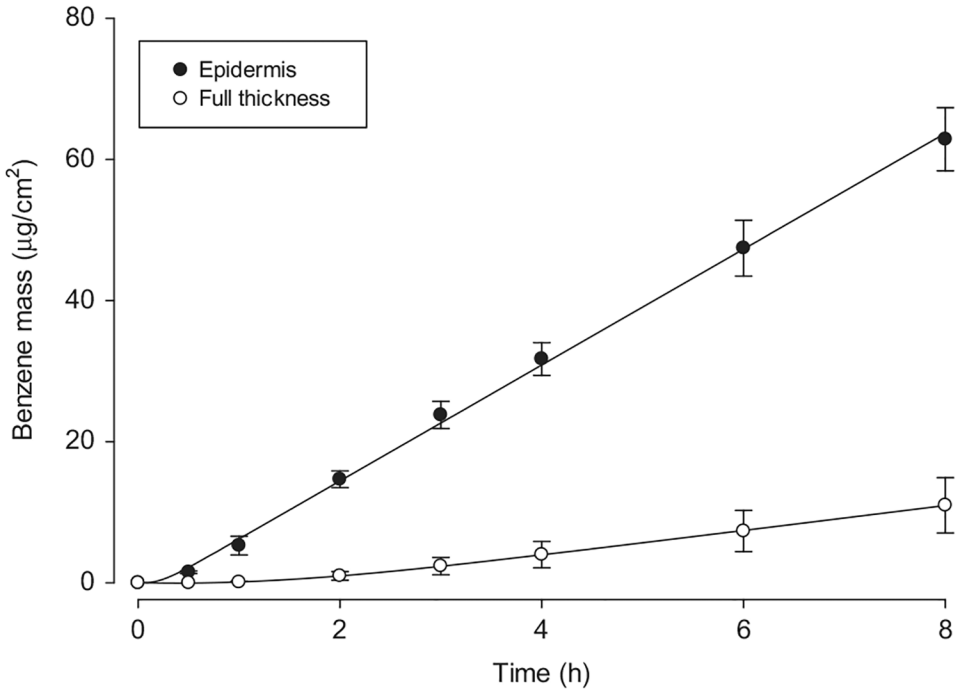

Figure 2.

Pooled permeation curves (mean ± SD, $n = 12$ each from same human donor) using epidermis and full-thickness skin exposed to infinite dose gasoline (0.8 vol% benzene). Solid lines are regressions of mean data with Eq. (3).

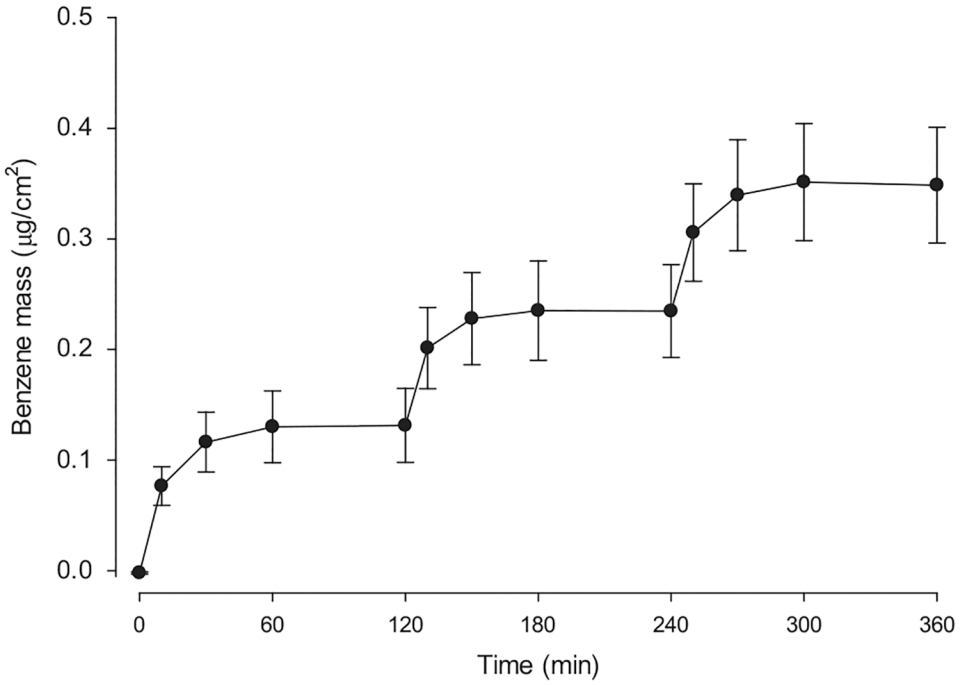

Figure 3.

Cumulative benzene permeation from three successive exposures to gasoline (1.8 vol% benzene) dosed at 10 μl/cm^2 (157 μg/cm^2 of benzene). Pooled (mean ± SD n = 12) data show accumulated mass detected in receptor compartments.

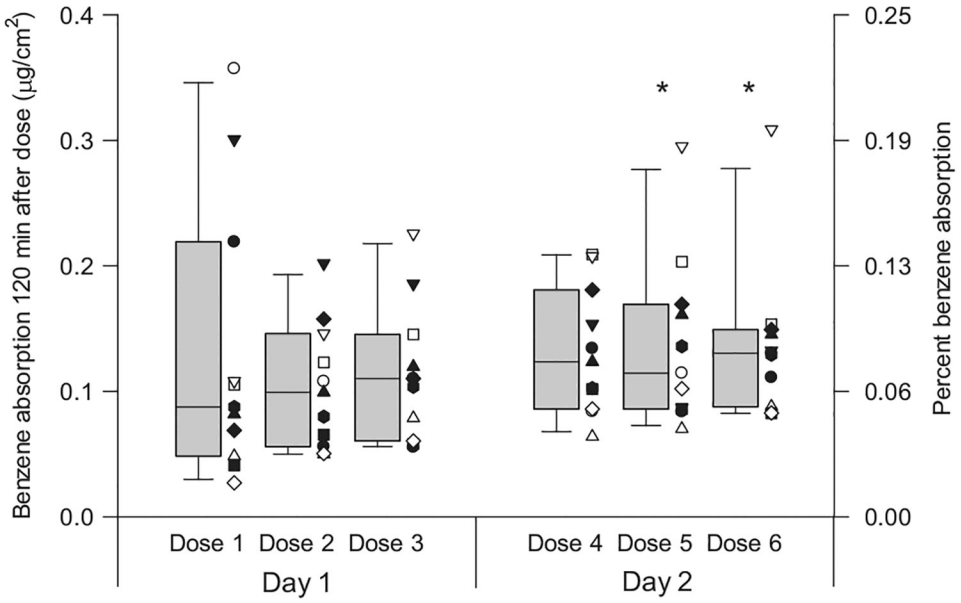

Figure 4.

Two-day, multiple-dose benzene permeation from gasoline dosed at 10 μl/cm^2 (157 μg/cm^2 benzene). Axes display mass of benzene detected in receptor fluid (left) and percentage of absorption of applied benzene dose (right) 120 min after dosing. Plots show differences in benzene absorption from previous exposure. Box plots show median, 25th and 75th percentiles and 10th and 90th percentiles. Scatter plots to the right of boxes show individual data points for all 12 skin samples. *Significant ($P < 0.05$) compared with Dose 1 (Friedman Repeated-Measures ANOVA). Also, median Day 1 values were less than median Day 2 values ($P < 0.05$, Mann–Whitney rank-sum test).

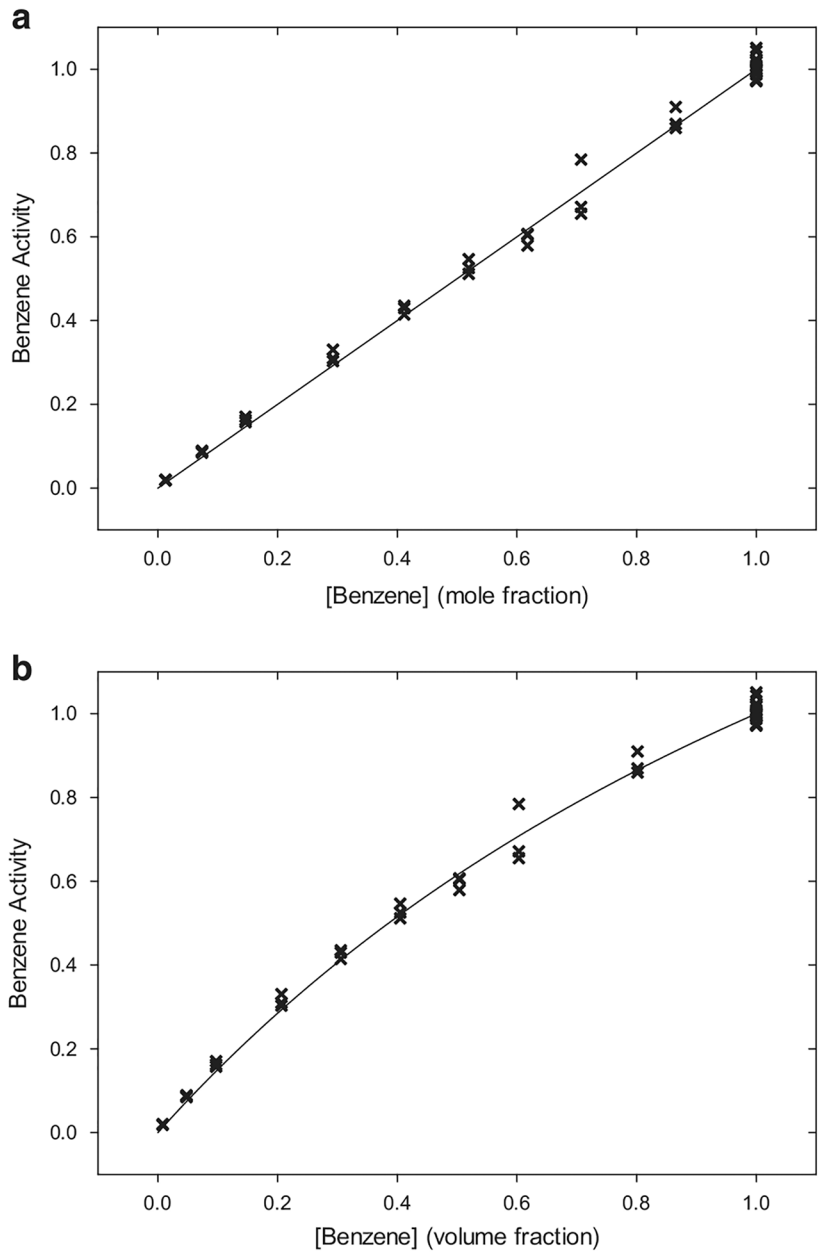

Figure 5.

Benzene thermodynamic activity in gasoline with benzene concentration as mole fraction (**a**) and as volume fraction (**b**). Each concentration was tested three times (nine times for neat benzene). Solid lines indicate ideal solution behavior.

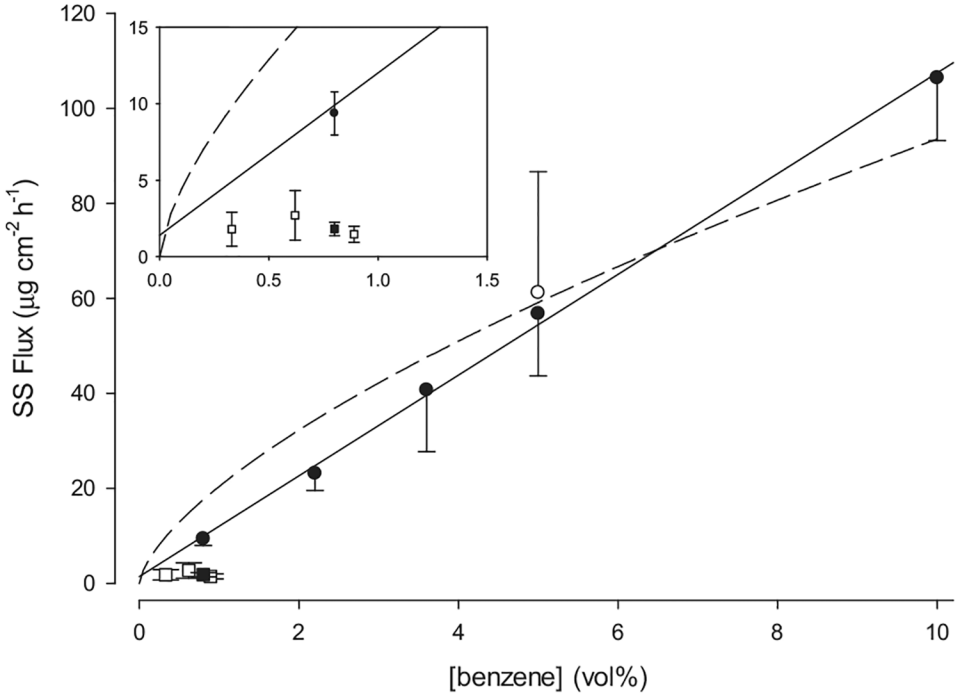

Figure 6.

Comparison of steady-state benzene flux measurements from gasoline among different studies. Solid circles represent data herein using human epidermis. White circle is data from Blank and McAullife[6] using human epidermis. Error bars (SD) are one-sided for clarity. Black square is data herein using full-thickness skin. White squares are data from Adami et al.[15] using full-thickness skin. Solid line is linear correlation (R^2 = 0.92) with our epidermis data (excluding neat benzene). Dashed line is the proposed relationship by Petty et al.[16] Inset is enlarged section of the plot.

Table 1.

Results of gasoline analysis undertaken by Bureau Veritas North America.

Method	Test	Result (Wt%/Vol%)
ASTM D5580	Benzene	0.93/0.78
	Toluene	6.56/5.57
	Ethylbenzene	2.34/1.99
	p/m-Xylenes	6.04/5.15
	o-Xylene	2.12/1.77
	C9+Aromatics	9.30/7.86
	Total Aromatics	27.29/23.12
ASTM D6730	Paraffins	5.477/6.370
	Isoparaffins	46.707/50.201
	Olefins	4.746/5.064
	Naphthenes	3.311/3.173
	Naphthalenes	0.212/0.152
	Aromatics	28.092/23.614
	C5 Hydrocarbons (HC)	9.210/10.742
	C14+HC	0.085/0.082
	Unknown	0.560/0.586
	Benzene	0.988/0.824
	Toluene	6.684/5.652
	Oxygenates	11.022/10.911

Concentrations of substances are listed according to the cited analytical method.

Table 2.

Skin permeation of benzene from gasoline through epidermis at different benzene concentrations.

[Benzene] (vol%/mg/ml)	J_{ss} (yg/cm^2/h)	k_p(10^{-3} cm/h)	t_{lag} (h)
0.80/6.99	9.37 ± 1.41 (10.2 ± 0.53)	1.34 ± 0.20 (1.46 ± 0.08)	0.12 ± 0.11 (0.25 ± 0.11)[a]
2.2/19.2	23.1 ± 3.54 (24.3 ± 3.64)	1.20 ± 0.18 (1.26 ± 0.19)	0.19 ± 0.15 (0.27 ± 0.11)
3.6/31.5	40.7 ± 13.0 (44.0 ± 14.1)	1.29 ± 0.41 (1.39 ± 0.44)	0.16 ± 0.08 (0.29 ± 0.14)[a]
5.0/43.7	58.7 ± 14.2 (61.3 ± 15.1)	1.34 ± 0.32 (1.40 ± 0.34)	0.24 ± 0.33 (0.25 ± 0.13)[a]
10/87.4	106 ± 13.2 (114± 12.7)	1.22 ± 0.15 (1.31 ± 0.14)	0.17 ± 0.10 (0.28 ± 0.12)[a]
100/874	566± 138	0.648 ± 0.16[b]	0.59 ± 0.15[b]

For each concentration, 3 samples from each of the 4 human donors were studied (n = 12). No significant differences in k_p and t_{lag} were found among gasoline+benzene formulations (one-way analysis of variance). Data in parentheses represent values derived from the first 4 h of the 8 h exposure time.

[a] $P < 0.05$ for 4 *vs* 8 h comparisons.

[b] $P < 0.05$ neat k_p and t_{lag} compared with all gasoline+benzene formulations.

J Expo Sci Environ Epidemiol. Author manuscript; available in PMC 2019 May 23.

Table 3.

Skin permeation of benzene from gasoline through epidermal membranes and full-thickness skin.

Skin type	J_{SS} (μg/cm^2/h)	k_p (10^{-3} cm/h)	t_{lag} (h)
Epidermis	8.22 ± 0.61	1.18 ± 0.09	0.25 ± 0.06
Full thickness	1.82 ± 0.44[a]	0.260 ± 0.06[a]	2.20 ± 1.01[a]

Twelve samples of both were studied from one human donor.

[a]Significantly ($P < 0.001$) different compared with epidermis (two-tailed t-test).